Exhibit 16

Exhibit 16

U.S. Patent No. 6,258,540: Patentable Subject Matter Requirement

Claim 1	Patentable Subject Matter Analysis
1. A method for detecting a paternally inherited nucleic acid of fetal origin performed on a maternal serum or plasma sample from a pregnant female, which method comprises	As of 1997, separating serum or plasma from blood to create a sample for nucleic acid analysis was a well-understood, routine, conventional activity previously engaged in by the scientific community (Leon et al. Cancer Res 1977; Shapiro et al. Cancer 1983; Vasioukhin et al. Br J Haematol 1994; Sorenson et al. Cancer Epi Biomarkers 1994; Chen et al. Nat Med 1996; Nawroz et al. Nat Med 1996). Indeed, the '540 patent specification refers to "standard techniques" for "preparation of serum or plasma from the maternal blood sample." (Ex. 2 at 2:26-27.) These standard techniques include using a centrifuge to separate the cells in the blood from the liquid component, followed by extracting (*i.e.*, purifying the DNA by removing proteins, often referred to as "isolating" the DNA) the DNA, sometimes using a commercial DNA extraction kit (*id.* at 4:38-51); controlled heating (*id.* at 2:31-32); and using an enzyme called "proteinase K" followed by either the chemicals phenol and/or chloroform to purify DNA from the sample (*id.* at 2:32-34). All of these techniques were well-understood, routine, and conventional as of 1997. There is no plasma or serum or DNA preparation technique disclosed in the '540 patent specification that was not well-understood, routine, and conventional as of 1997. The '540 patent does not disclose any newly identified "paternally inherited nucleic acid." There were many paternally inherited nucleic acid sequences that were known in 1997, such as sequences on the Y chromosome (which could not be maternally inherited because only males possess a Y chromosome). (*See, e.g.*, *id.* at 7:1-3 ("Sequence data for the SRY gene were obtained from the GenBank Sequence Database (accession number L08063).").) Furthermore, it was well-understood at that time that a fetus inherits half of its DNA from its mother and half from its father, and that the father's nucleic acid sequences, *i.e.*, nucleic acid sequences that could be paternally inherited, could be determined through a process known as "genotyping." (*See, e.g.*, *id.* at 3:20-24 ("This application will require the prior genotyping of the father and mother using a panel of polymorphic markers and then

	an allele for detection will be chosen which is present in the father, but is absent in the mother.").) Also, by 1997, scientists had access to publicly available DNA sequences through the GenBank sequence database. Many techniques for detecting a paternally inherited nucleic acid of fetal origin were well-understood, routine, and conventional as of 1997. These included gel electrophoresis, which often involves passing an electric current through a rectangular piece of porous "gel" material. The gel is usually made of a polymerized matrix of agarose or polyacrylamide. The nucleic acid sample is placed at the top of the gel, and the electric current causes the nucleic acid molecules to move through the gel in a fashion dependent on their size. Larger molecules move more slowly than smaller molecules. Thus the molecules begin to separate by size. Once there is sufficient separation, the electric current is switched off. The nucleic acid molecules, which are not visible to the human eye, are then subjected to a "staining" procedure, so that they can be seen in the gel. Ethidium bromide is a chemical often used to stain nucleic acid molecules in a gel, because it binds to the nucleic acid molecules. Moreover, it will cause the nucleic acid molecules to fluoresce upon exposure to ultraviolet light. Real time quantitative PCR ("Q-PCR") was another well-understood, routine, and conventional method of detecting nucleic acid in 1997. In this technique, nucleic acid is amplified using PCR and the amplified nucleic acid is quantified in real time, *i.e.*, as the amplification is taking place, generally through the use of a fluorescent dye that attaches to the newly generated nucleic acid. Gel electrophoresis and Q-PCR are disclosed in the '540 patent as methods for "detecting" paternally inherited nucleic acids. (*See, e.g.*, *id.* at 5:23-25 ("PCR products were analysed by agarose gel electrophoresis and ethidium bromide staining."); 6:37-40 ("Real time quantitative PCR analysis was performed using a PE Applied Biosystems 7700 Sequence Detector (Foster City, Calif., U.S.A.).").) All of the detection methods disclosed in the '540 patent specification were well-understood, routine, and conventional as of 1997.
amplifying a paternally inherited nucleic acid from the serum or plasma sample and	The specification acknowledges that "[s]tandard nucleic acid amplification systems can be used" to amplify fetal nucleic acid in the serum or plasma sample, "including PCR, the ligase chain reaction, nucleic acid sequence based amplification (NASBA), branched DNA methods, and so on." (Ex. 2 at 2:43-48.) PCR is an acronym for the

	polymerase chain reaction. In this amplification method, DNA, which is double stranded, is separated into two strands using heat, which disrupts the bonds between the nucleotides on each strand. Next, enzymes are used along with short DNA fragments that are necessary to begin the amplification process (called "primers"), and free nucleotides to build two new strands of DNA that complement the old strands (often referred to as "template" strands). The process is then repeated, starting with separation of the strands of all DNA molecules in the mixture—both old and new—through a heating step, followed by the priming and amplification step, resulting in essentially geometric amplification of the DNA target sequences. The ligase chain reaction is similar to PCR in that it uses cycles of separating the DNA through heating and amplifying the DNA using enzymes. However, two primers are used that bond to adjacent locations on a strand of DNA, and then an enzyme known as a ligase bonds the two primers together. Nucleic acid sequence based amplification (NASBA) is a technique for amplifying RNA using primers, nucleotides, and enzymes at a constant temperature. Branched DNA methods involve the use of a number of short DNA fragments that interact with the target nucleic acid. But branched DNA methods do not increase the number of copies of the target nucleic acid. Instead, they are used for detection of a target nucleic acid. Regardless of the approach listed, all of these methods were well-understood, routine, and conventional as of 1997. There is no amplification method disclosed in the '540 patent specification that was not well-understood, routine, and conventional as of 1997.
detecting the presence of a paternally inherited nucleic acid of fetal origin in the sample.	Many techniques for doing this were well-understood, routine, and conventional as of 1997. These included gel electrophoresis, which often involves passing an electric current through a rectangular piece of porous "gel" material. The gel is usually made of a polymerized matrix of agarose or polyacrylamide. The nucleic acid sample is placed at the top of the gel, and the electric current causes the nucleic acid molecules to move through the gel in a fashion dependent on their size. Larger molecules move more slowly than smaller molecules. Thus the molecules begin to separate by size. Once there is sufficient separation, the electric current is switched off. The nucleic acid molecules, which are not visible to the human eye, are then subjected to a "staining" procedure, so that they can be seen in the gel. Ethidium bromide is a chemical often used to stain nucleic acid molecules in a gel, because it binds to the nucleic acid

	molecules. Moreover, it will cause the nucleic acid molecules to fluoresce upon exposure to ultraviolet light. Real time quantitative PCR ("Q-PCR") was another well-understood, routine, and conventional method of detecting nucleic acid in 1997. In this technique, nucleic acid is amplified using PCR and the amplified nucleic acid is quantified in real time, *i.e.*, as the amplification is taking place, generally through the use of a fluorescent dye that attaches to the newly generated nucleic acid. Gel electrophoresis and Q-PCR are disclosed in the '540 patent as methods for "detecting" paternally inherited nucleic acids. (*See, e.g.*, Ex. 2 at 5:23-25 ("PCR products were analysed by agarose gel electrophoresis and ethidium bromide staining."); 6:37-40 ("Real time quantitative PCR analysis was performed using a PE Applied Biosystems 7700 Sequence Detector (Foster City, Calif., U.S.A.).").) All of the detection methods disclosed in the '540 patent specification were well-understood, routine, and conventional as of 1997. Amplifying and detecting cell-free nucleic acid in a plasma or serum sample were routinely used together as of 1997. For example, amplifying and detecting cell-free nucleic acid in plasma or serum samples was a technique that was already being used for tumor DNA by 1994 (Vasioukhin et al. Br J Haematol 1994; Sorenson et al. Cancer Epi Biomarkers 1994) and subsequently in 1996 (Chen et al. Nat Med 1996; Nawroz et al. Nat Med 1996), as acknowledged in the '540 patent specification (Ex. 2 at 1:40-43 ("[I]t has been demonstrated that tumour DNA can be detected by the polymerase chain reaction (PCR) in the plasma or serum of some patients (Chen et al 1996; Nawroz et al 1996).")). Thus, it is my opinion that the steps of claim 1, when viewed as a whole, add nothing significant beyond the sum of their parts taken together.
Claim 2	Patentable Subject Matter Analysis
2. The method according to claim 1, wherein the foetal nucleic acid is amplified by the polymerase chain reaction.	As discussed above, PCR was a well-understood, routine, and conventional process as of 1997. Even the particular PCR techniques discussed in the '540 patent were well-understood, routine, and conventional as of 1997.
Claim 8	Patentable Subject Matter Analysis
8. The method according to claim 1, wherein the presence	As of 1997, there were many well-understood, routine, and conventional methods for the amplification and detection of

of a foetal nucleic acid from a paternally-inherited non-Y chromosome is detected.	fetal nucleic acid from a paternally inherited non-Y chromosome. Indeed, the '540 patent itself references several: Camaschella et al. Blood 1990; Lo et al. Lancet 1993; Bennett et al. N Engl J Med 1993; Bianchi et al. Am J Obstet Gyn 1994; Geifman-Holtzman et al. Am J Obstet Gyn 1996; Aubin et al. Br J Hematol 1997. Furthermore, it was well-understood at that time that a fetus inherits half of its DNA from its mother and half from its father, and that the father's non-Y chromosome nucleic acid, *i.e.*, non-Y chromosome nucleic acid that could be paternally inherited, could be determined through a process known as "genotyping." (*See, e.g.*, Ex. 2 at 3:20-24 ("This application will require the prior genotyping of the father and mother using a panel of polymorphic markers and then an allele for detection will be chosen which is present in the father, but is absent in the mother.").) Moreover, a number of non-Y chromosome nucleotide sequences were available through the GenBank sequence database.
Claim 19	Patentable Subject Matter Analysis
19. The method according to claim 1, wherein the sample contains foetal DNA at a fractional concentration of total DNA of at least about 0.14%, without subjecting it to a foetal DNA enrichment step.	It is a law of nature that cell-free fetal DNA is present in the serum or plasma from a pregnant female and that the fractional concentration of fetal DNA to total DNA in maternal plasma or serum may vary within an individual pregnant female over time and among pregnant females (Kazakov et al. Tsitologiia 1995; Lo et al. Am J Hum Genet 1998; Zhong et al. Obstet Gynecol 2000; Smid et al. Ann NY Acad Sci 2001; Bauer et al. Prenatal Diag 2006). As a result of natural human physiological processes, the fractional concentration of fetal DNA to total DNA in serum or plasma of pregnant women far exceeds 0.14%, at least from late in the first trimester onwards (Lun et al. Clin Chem 2008; Lo Clin Chem Lab Med 2012; Lo et al. Am J Hum Genet 1998). This is far in excess of the fraction recited in claim 19. The parameter set forth in claim 19 is therefore a law of nature in and of itself. The human body naturally and necessarily produces far in excess of 0.14% fractional concentration of fetal DNA to total DNA in serum or plasma during pregnancy.
Claim 20	Patentable Subject Matter Analysis
20. The method according to claim 19, wherein the	It is a law of nature that cell-free fetal DNA is present in the serum or plasma from a pregnant female and that the

fractional concentration of foetal DNA is at least about 0.39%.	fractional concentration of fetal DNA to total DNA in maternal plasma or serum may vary within an individual pregnant female over time and among pregnant females (Kazakov et al. Tsitologiia 1995; Lo et al. Am J Hum Genet 1998; Zhong et al. Obstet Gynecol 2000; Smid et al. Ann NY Acad Sci 2001; Bauer et al. Prenatal Diag 2006). As a result of natural human physiological processes, the fractional concentration of fetal DNA to total DNA in serum or plasma of pregnant women far exceeds 0.39%, at least from late in the first trimester onwards (Lun et al. Clin Chem 2008; Lo Clin Chem Lab Med 2012; Lo et al. Am J Hum Genet 1998). This is far in excess of the fraction recited in claim 20. The parameter set forth in claim 20 is therefore a law of nature in and of itself. The human body naturally and necessarily produces far in excess of 0.39% fractional concentration of fetal DNA to total DNA in serum or plasma during pregnancy.
Claim 21	Patentable Subject Matter Analysis
21. A method of performing a prenatal diagnosis, which method comprises the steps of:	The specified method of performing a prenatal diagnosis in claim 21 relies entirely on well-understood, routine, and conventional methodologies as of 1997, coupled to the law of nature discussed extensively above–*i.e.*, the existence of cell-free fetal nucleic acid in the serum or plasma from a pregnant female. No novel or inventive concepts, processes, or methodologies are specified in claim 21.
(i) providing a maternal blood sample;	Blood samples have been collected by doctors long before 1997. Thus, the step of "providing a maternal blood sample" was well-understood, routine, and conventional by 1997. There is no disclosure in the '540 patent specification of any methods for "providing a maternal blood sample" that were not well-understood, routine, and conventional as of 1997.
(ii) separating the sample into a cellular and a non-cellular fraction;	A "cellular" fraction means a portion of the sample that contains cells. A "non-cellular" fraction means a portion of the sample that does not contain cells. Both plasma and serum are non-cellular fractions of a blood sample because, by definition, neither contains cells. Separating blood from a maternal blood sample into cellular and non-cellular (*i.e.*, plasma or serum) fractions was a well-understood, routine, and conventional activity for researchers in the field by

	1997, as I previously detailed in my analysis of claim 1.
(iii) detecting the presence of a nucleic acid of foetal origin in the non-cellular fraction according to the method of claim1;	As detailed above in comments on claim 1, this was a well-understood, routine, and conventional activity for researchers in the field by 1997.
(iv) providing a diagnosis based on the presence and/or quantity and/or sequence of the foetal nucleic acid.	Prenatal diagnosis as defined in the '540 patent "covers determination of any maternal or foetal condition or characteristic which is related to either the foetal DNA itself or the quantity or quality of the foetal DNA in the maternal serum or plasma." (Ex. 2 at 2:6-10.) The '540 patent specification does not disclose any newly discovered "foetal nucleic acid" whose presence and/or quantity and/or sequence has diagnostic significance. Instead, the specification points to well established diagnostic measures: > We envisage that foetal DNA analysis in maternal plasma and serum would be most useful in situations where the determination of foetal-derived paternally-inherited polymorphisms/mutations or genes would be helpful in clinical prenatal diagnosis (Lo et al. 1994). Examples include foetal sex determination for the prenatal diagnosis of sex-linked disorders, foetal rhesus D status determination in sensitized rhesus negative pregnant women (Lo et al. 1993), autosomal dominant disorders in which the father carries the mutation and autosomal recessive genetic disorders in which the father and mother carry different mutations (Lo et al.1994), e.g., certain hemoglobinopathies (Camaschella et al. 1990) and cystic fibrosis. (*Id.* at 17:35-47.) The claim is broadly directed to providing a diagnosis based on analysis of fetal nucleic acid, which doctors have been doing for many years. For example, prior publications referenced in the '540 patent had already demonstrated the use of nucleic acid amplification and detection methods for prenatal diagnostic approaches on nucleic acid of fetal origin from maternal blood (Lo et al. Lancet 1990; Camaschella et al. Blood 1990; Bennett et al. N Engl J Med 1993; Lo et al. Lancet 1993; Lo et al. Ann NY Acad Sci

	1994; Bianchi et al. Am J Obstet Gyn 1994; Bianchi et al. Ped Res 1996; Bianchi et al. Am J Hum Genet 1997; Aubin et al. Br J Hematol 1997), including prenatal diagnosis approaches based on the presence and/or quantity and/or sequence of nucleic acid of fetal origin obtained from fetal cells in the maternal circulation (reviewed in Steele et al. Clin Obstet Gynecol 1996).
Claim 22	Patentable Subject Matter Analysis
22. The method according to claim 21, wherein the non-cellular fraction as used in step (iii) is a plasma fraction.	Claim 22 specifies that the sample is plasma, as opposed to serum. All of the contemplated techniques for preparing and handling plasma were well-understood, routine, and conventional as of 1997. I discuss this state of the art at length in my analysis of claim1.
Claim 24	Patentable Subject Matter Analysis
24. A method for detecting a paternally inherited nucleic acid on a maternal blood sample, which method comprises:	Like claim 1, claim 24 is "[a] method for detecting a paternally inherited nucleic acid," which requires "amplifying a paternally inherited nucleic acid" and "subjecting the amplified nucleic acid to a test for the [p]aternally inherited fetal nucleic acid." As detailed above in comments on claim 1, by 1997, these were well-understood, routine, and conventional activities performed by researchers in the field.
removing all or substantially all nucleated and anucleated cell populations from the blood sample,	Nucleated cells are cells that contain a nucleus. Most human cells are nucleated. Anucleated cells are cells that do not contain a nucleus. Some cell types in the human body normally lack a nucleus, such as normal adult red blood cells, whereas others may lack a nucleus as a result faulty cell division. Both plasma and serum are, by definition, free of substantially all nucleated and anucleated cells. As discussed in my analysis of claim 1, as of 1997, there were a number of well-understood, routine, and conventional techniques for "removing all or substantially all nucleated and anucleated cell populations from [a] blood sample," *i.e.*, preparing plasma and serum samples. The patent specification does not reveal that any new techniques that were contemplated. Furthermore, when performing a test to detect nucleic acid in plasma or serum, it was well-understood, routine, and conventional for a researcher to prepare samples that removed all nucleated and anucleated cell populations from the blood sample (Mandel & Metais Proc Meetings Biol Soc 1948; Tan et al. J Clin Invest 1966; Kamm & Smith Clin Chem 1972; Leon et al. Cancer Res

	1977; Shapiro et al. Cancer 1983; Emanuel & Pestka Gen Anal Tech Appl 1993; Vasioukhin et al. Br J Haematol 1994; Sorenson et al. Cancer Epi Biomarkers 1994; Fowke et al. J Immunol Methods 1995; Chen et al. Nat Med 1996; Nawroz et al. Nat Med 1996).
amplifying a paternally inherited nucleic acid from the remaining fluid and subjecting the amplified nucleic acid to a test for the Paternally inherited fetal nucleic acid.	Like claim 1, claim 24 is "[a] method for detecting a paternally inherited nucleic acid," which requires "amplifying a paternally inherited nucleic acid" and "subjecting the amplified nucleic acid to a test for the [p]aternally inherited fetal nucleic acid." While this claim refers to "subjecting the amplified nucleic acid to a test for the [p]aternally inherited fetal nucleic acid," there is nothing in the patent specification that suggests that this is any different than "detecting a paternally inherited nucleic acid" as appears earlier in the claim and in claim 1. As detailed above in comments on claim 1, by 1997, these were well-understood, routine, and conventional activities performed by researchers in the field.
Claim 25	Patentable Subject Matter Analysis
25. A method for performing a prenatal diagnosis on a maternal blood sample, which method comprises	As I explained in my analysis of claim 21, doctors had been performing prenatal diagnosis on maternal blood samples for many years prior to 1997.
obtaining a non-cellular fraction of the blood sample	As discussed earlier, there were many well-understood, routine, and conventional ways to do this by 1997. The patent specification does not reveal any new ways of obtaining a non-cellular fraction of a maternal blood sample. Furthermore, when performing analysis of nucleic acid in plasma or serum, it was well-understood, routine, and conventional for a researcher to prepare samples that removed the cellular fraction from the blood sample (Vasioukhin et al. Br J Haematol 1994; Sorenson et al. Cancer Epi Biomarkers 1994; Fowke et al. J Immunol Methods 1995; Chen et al. Nat Med 1996; Nawroz et al. Nature Med 1996).
amplifying a paternally inherited nucleic acid from the non-cellular fraction	For the reasons stated in the analysis of claim 1, this was a well-understood, routine, and conventional activity performed by researchers in the field as of 1997.
and performing nucleic acid analysis on the amplified nucleic acid to detect paternally inherited fetal	While this claim refers to "performing nucleic acid analysis . . . to detect paternally inherited fetal nucleic acid," there is nothing in the patent specification that suggests that this is any different than the "detecting" step in claim 1. For the

nucleic acid.	reasons stated in the analysis of claim 1, this was a well-understood, routine, and conventional activity performed by researchers in the field as of 1997.